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Effects of dietary oregano powder supplementation on the growth performance, antioxidant status and meat quality of broiler chicks

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ABSTRACT

A 6-week experiment was conducted to evaluate the effects of dietary oregano powder (OP) supplementation on the growth performance, antioxidant status and meat quality of broiler chicks. A total of 180 one day-old Arbor Acres broilers were randomly divided into 3 treatments with 6 replicates and 10 chicks per replicate. The chicks were fed with basal diet without (CTR), or with 20 mg/kg of virginiamycin (ATB), or with 150 mg/kg of OP. At 21 and 42 days of age, two birds from each cage were selected for sampling. Compared to the CTR group, the OP supplementation increased average daily gain and average daily feed intake during the grower period ($p = .047$ and $.03$, respectively) and the whole period ($p = .04$ and $.02$, respectively). The supplementation of ATB and OP did not affect the immune organ index of chicks. In addition, dietary OP reduced malondialdehyde content and increased total antioxidant activity (T-AOC) in the serum of chicks at 21 ($p < .01$) and 42 ($p < .01$) days of age, and chicks fed OP had higher T-AOC than the ATB chicks at 21 days of age ($p < .01$). However, no dietary effect was observed on carcass yield, cooking loss, dripping loss, shear force, pH value and meat colour. The results of the present study indicate that dietary OP supplementation could positively improve the growth and systemic antioxidative defence property of broiler chicks, which had potential to act as a growth promoter comparable to antibiotic in broiler chicks.

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Introduction

Nowadays, many phyto-genic sources have been investigated and tested as alternatives to chemical antibiotics for poultry and livestock (Hashemi & Davoodi 2010). Especially, after the use of chemical antibiotics as growth promoters was banned in Europe, research about phyto-genic source to replace chemical antibiotics has been rapidly growing worldwide (Windisch et al. 2008). Chemical antibiotics used to be approved as growth promoters from the middle of the last century (Castanon 2007); however, concerns about their residue (Vondruskova et al. 2010) and antibiotic-resistant bacteria (Van der Fels-Klerx et al. 2011) finally resulted in the ban on using these chemical substances (Karimi et al. 2010).

Many phyto-genic sources to replace antibiotics show promising results not only as an antimicrobial

agent (Wong et al. 2008) but also in other respects such as antioxidant ability and growth promoter function (Krishan & Narang 2014; Zeng et al. 2015). Oregano (*Origanum vulgare* L.) is an aromatic herb containing abundant active chemical compounds (Falco et al. 2013; Park et al. 2015), which has been used to replace chemical antibiotics in poultry and livestock (Ertas et al. 2005) and its effect as a feed additive has been investigated in many previous studies. The major components of oregano essential oil are terpenoid compounds such as carvacrol and thymol (Teixeira et al. 2013; Krishan & Narang 2014). As a secondary metabolite, the antimicrobial activity of the polyphenols and its molecular mechanism to suppress microbes was reported and discussed in previous research (Ben Arfa et al. 2006; Xu et al. 2008). Moreover, the extract from oregano, as a natural

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antioxidant, has a pronounced ability to prevent lipid oxidation (Botsoglou et al. 2003; Lee et al. 2004), which contributes to meat quality and animal's health.

Although oregano has shown high potential from its chemical nature to improve animal health and growth, the previous research mainly concentrated on effects of oregano powder on a few specific physiological parameters and few studies about meat quality have been conducted so far to decide on the commercial value of the meat production. Therefore, the objective of this study was to evaluate the efficacy of dietary OP supplementation on the growth performance, immune and antioxidant status, as well as meat quality of modern fast-growing broiler chicks.

Materials and methods

The animal protocol for this research was approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

Animals, diets and management

The experiment was conducted in Nan Kou pilot base of the Chinese Academy of Agricultural Sciences during fall-winter season in 2015. A total of 180 day-old Arbour Acres broiler chicks (half males and half females) were randomly divided into three treatments that were further allocated to six replicates. Each replicate consisted of 10 broilers that were housed in cages. The dietary groups were as follows: the control group (CTR) that was fed a basal diet without additive, the ATB group that was fed a 20 mg of chemical antibiotic (virginiamycin)/kg diet and the OP group that was fed with 150 mg/kg of oregano powder (Meriden Animal Health Ltd., Luton, UK). The basal diet was a typical corn-soybean diet formulated to meet the nutrient requirements of broiler chicks (NRC 1994), for starter chicks from 1 to 21 days and for grower chicks from 22 to 42 days of age. The diet during the starter period was provided in crumble form, and the diet during the grower period was provided as pellets. The compositions of the basal diets and nutrient levels are presented in Table 1. Determination of crude protein, calcium and phosphorus were performed using the Association of Analytical Communities (AOAC 2005) official method AOAC 2001.11, AOAC 927.02 and AOAC 964.06, respectively. Feed and water were provided *ad libitum* at all times during the experimental period. The management of the birds was in accordance with the guidelines of raising Arbour Acres broilers. The birds were raised in wire floor cages

Table 1. Ingredients and nutrient composition of basal diets (air-dry basis).

Item	Starter (day 1 to 21)	Grower (day 22 to 42)
Ingredient, %		
Maize	56.68	60.37
Soybean meal	31.86	27.32
Rapeseed meal	2.50	2.50
Cottonseed meal	2.00	2.50
Soybean oil	2.78	3.57
Dicalcium phosphate	1.81	1.51
Limestone	1.28	1.22
NaCl	0.35	0.35
DL-Methionine	0.21	0.12
L-Lysine hydrochloride	0.12	0.13
Vitamin premix ^a	0.02	0.02
Trace element premix ^b	0.20	0.20
Choline chloride	0.10	0.10
Nutrient content ^c		
AME ^d , MJ/kg	12.35	12.77
Crude protein, %	20.50 (20.48)	19.00 (18.96)
Calcium, %	1.00 (0.98)	0.90 (0.91)
Phosphorous, %	0.71 (0.69)	0.64 (0.63)
Available phosphorous, %	0.45	0.40
Lysine, %	1.10	1.00
Methionine, %	0.49	0.39
Methionine + cysteine, %	0.81	0.69

^aVitamin premix (per kilogram of feed): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 18.75mg; vitamin K3, 2.65mg; vitamin B1, vitamin B2, vitamin B12 0.025mg; biotin, 0.0325mg; folic acid, 1.25mg; pantothenic acid, 12mg; niacin, 50mg.

^bCu, 8ppm; Zn, 75ppm; Fe, 80ppm; Mn, 100ppm; Se, 0.15ppm; I, 0.35ppm.

^cThe nutrient levels listed in parentheses are analysed values, others are calculated ones.

^dAME value was estimated from Sauvant et al. (2004).

(cage size: 100 cm × 90 cm × 65 cm³) in a four-level battery in an environmentally controlled room under continuous incandescent white light, and the relative humidity was kept between at 65 and 70%. The temperature of the chicken house was 31 °C for the first 2 weeks, and then gradually decreased to 25 °C over the next two weeks and for the remaining time was kept at 25 °C.

Growth performance parameters

Average daily weight gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for the starter (1 to 21 day), grower (22 to 42 day) and the whole period of the experiment (1 to 42 day).

Immune organ index and serum antioxidant activity

At 21 and 42 days of age, two birds from each cage, close to the cage average weight, were selected and slaughtered by cutting the jugular vein. Blood samples were collected in heparinised test tubes during bleeding to analyse the plasma antioxidant differences among dietary treatments and were immediately

centrifuged at $3000 \times g$ for 10 min at 4°C (TL6R, Hunan Herexi Instrument & Equipment Co. LTD., Hunan, China) to separate the serum. Then, the serum samples were stored at -20°C until analysis. The serum within the replicate was pooled and analysed in duplicate.

At 21 and 42 days of age, the lymphoid organs (thymus, spleen and bursa) of chicks were collected, weighed and their relative weights were calculated using the following formula:

$$I_t = W_t/W_g; I_s = W_s/W_g; I_b = W_b/W_g;$$

W_t : weight of thymus (g); W_s : weight of spleen (g); W_b : weight of bursa (g); W_g : gross weight of the sample (kg).

In order to assess the antioxidant activity of the serum samples, the analysis of malondialdehyde (MDA), total antioxidant activity (T-AOC) and superoxide dismutase (SOD) was performed using a spectrophotometric procedure (Versa Max, Molecular Devices Shanghai Corporation, Shanghai, China) with the commercial assay kits (Nanjing Jiancheng Biological Engineering Research Institute, Nanjing, China). The MDA concentration was analysed with 2-thiobarbituric acid, and the change in absorbance was read at 532 nm. The T-AOC was measured by a ferric reducing/antioxidant power assay (Benzie & Strain 1996). Activity of SOD was calculated through nonenzymatic NBT test, which measures the inhibition of formation of superoxide anion free radicals that reduce the nitroblue tetrazolium of the sample (Winterbourn et al. 1975).

Meat quality parameters

At 42 days of age, the breast and thigh muscle were removed and weighed, and the ratio of the muscles to the eviscerated carcass was calculated.

$$R_{bm} = w_{bm}/w_e; R_{tm} = w_{tm}/w_e;$$

w_{bm} : weight of breast muscle; w_{tm} : weight of thigh muscle; w_e : weight of eviscerated carcass.

To assess the water holding capacity of the meat, a left breast muscle weighing 30 ± 1 g was removed within 45 min after slaughtering. The sample was preserved at 4°C in a refrigerator for 24 h and then was weighed again to assess the water holding capacity. Then, the sample was placed into a plastic bag and cooked in a thermostatic bath (DK-S24, Shanghai pot department scientific instrument Co. LTD., Shanghai, China) at 80°C for 10 min, then left under running water and equilibrated at room temperature. The sample was weighed again to estimate the percentage of

cooking loss (%). After testing the cooking loss, the cooked meat was perpendicularly divided into 2 strips that were 2.0-cm long, 1.0-cm wide and 0.5-cm thick, respectively. Shear force was assessed using a tenderness metre (C-LM3B, Tenovo, China) with a 25 kg load cell and a crosshead speed of 300 mm/min. As important meat quality parameters, pH and meat colour were measured just after slaughtering and 24 h later. The breast muscle pH was tested (three measurements per sample) at a depth of 2.5 cm below the surface using a waterproof spear-type pocket pH metre (pH Spear, Eutech Instruments Pte Ltd., Ayer Rajah Crescent, Singapore). The pH metre was calibrated with two buffers at pH 4.0 and 7.0 (Merck, Darmstadt, Germany) at ambient temperature. The complete CIE system colour profile of lightness (L^*), redness (a^*), and yellowness (b^*) was measured (three measurements per sample) in the same site in which the pH was measured using a colorimeter (Chroma Metre WSC-S, Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China). White and black tiles were used as standards. The colorimeter had the illumination geometric condition 0/d, combined CIE standard illuminant D65 with 10 wide viewing field X10 Y10 and Z10, and possessed the measuring area from $\varnothing 2.5$ - $\varnothing 30$.

Statistical analysis

All the experimental data were analysed by one-way ANOVA using the SPSS analysis software package programme (SPSS 21.0, SPSS Inc., Chicago, IL). The model included the treatment effect, and the cage represented the experimental unit for growth performance, while the bird was the experimental unit for meat quality, immune and antioxidant parameters. The treatment comparisons were performed using Tukey's honestly significant difference test for multiple testing. The treatment effects were considered significant at $p \leq .05$, whereas a trend for a treatment effect was noted for $p \leq .10$.

Results

The effects of the dietary oregano powder supplementation on growth performance of broilers in different growth periods are presented in Table 2. No dietary effect was observed on the growth performance from day 1 to day 21 ($p > .05$). Compared to the CTR group, the supplementation of OP increased ADG and ADFI during the grower period ($p = .047$ and $.03$, respectively) and tended to increase the ADG and ADFI during the overall experiment period ($p = .04$ and $.02$,

Table 2. Effects of dietary oregano powder supplementation on growth performance of broiler chicks^c.

Items	CTR	ATB	OP	SEM	p-value
Day 1 to 21					
ADG, g/d	39.74	41.53	39.56	0.87	.26
ADFI, g/d	54.73	58.66	56.24	1.55	.23
FCR	1.38	1.41	1.42	0.03	.56
Day 22 to 42					
ADG, g/d	71.84 ^y	80.21	81.84 ^x	2.74	.047
ADFI, g/d	134.58 ^b	148.26 ^a	148.20 ^a	3.59	.03
FCR	1.88	1.85	1.82	0.03	.40
Day 1 to 42					
ADG, g/d	54.85 ^y	60.15 ^x	59.47 ^x	1.46	.04
ADFI, g/d	92.28 ^{b,y}	101.79 ^a	99.64 ^x	2.11	.02
FCR	1.68	1.69	1.68	0.01	.67

ATB: antibiotic; CTR: basal diet without additive; OP: oregano powder; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio.

^{a,b}Means listed in the same row with different superscripts are significantly different ($p \leq .05$).

^{x,y}Means listed in the same row with different superscripts are tended to be different ($p \leq .10$).

^c $n = 6$ replicates/treatment.

Table 3. Effects of dietary oregano powder supplementation on immune organ index of broiler chicks^a.

Items	CTR	ATB	OP	SEM	p-value
Day 21					
I_t , g/kg	3.26	2.30	2.74	0.37	.26
I_s , g/kg	0.96	1.10	0.99	0.11	.66
I_b , g/kg	1.93	1.74	1.73	0.19	.73
Day 42					
I_t , g/kg	2.59	2.24	2.27	0.19	.39
I_s , g/kg	1.92	1.78	1.81	0.16	.82
I_b , g/kg	0.79	0.57	0.64	0.08	.19

ATB: antibiotic; CTR: basal diet without additive; OP: oregano powder; I_t : weight of thymus (g)/gross weight of the sample (kg); I_s : weight of spleen (g)/gross weight of the sample (kg); I_b : weight of bursa (g)/gross weight of the sample (kg).

^a $n = 6$ replicates/treatment.

respectively), and dietary ATB increased ADFI during the grower and overall experiment periods ($p = .03$ and $.02$, respectively) and tended to increase ADG from day 1 to day 42 ($p = .04$). However, no significant difference between the ATB and OP groups was observed in the growth performance of chicks ($p > .05$).

Tables 3 and 4 show the effects of the dietary oregano powder supplementation on immune organ index and serum antioxidant activity of broiler chicks. As can be seen, no dietary effect on the index of the lymphoid organ of chicks at 21 and 42 days of age ($p > .05$). Dietary OP significantly reduced MDA content and increased T-AOC compared to the CTR group at 21 ($p < .01$) and 42 ($p < .01$) days of age. Supplementation of ATB decreased the MDA level compared to the CTR group at both 21 and 42 days of age ($p < .01$). In addition, chicks fed OP had higher T-AOC than the ATB chicks at 21 days of age ($p < .01$) and tended to increase T-AOC compared to the ATB group at 42 days of age ($p < .01$). However, SOD

Table 4. Effects of dietary oregano powder supplementation on serum antioxidant activity of broiler chicks^c.

Items	CTR	ATB	OP	SEM	p-value
Day 21					
MDA, nmol/mL	3.40 ^a	2.12 ^b	2.10 ^b	0.18	<.01
T-AOC, U/mL	11.69 ^b	7.13 ^b	20.52 ^a	2.01	<.01
SOD, U/mL	152	204	231	32	.24
Day 42					
MDA, nmol/mL	5.06 ^a	2.84 ^b	2.52 ^b	0.20	<.01
T-AOC, U/mL	2.47 ^b	6.27 ^y	11.46 ^{a,x}	1.41	<.01
SOD, U/mL	233	279	288	27	.41

ATB: antibiotic; CTR: basal diet without additive; OP: oregano powder; MDA: malondialdehyde; T-AOC: total antioxidant capacity; SOD: superoxide dismutase.

^{a,b}Means listed in the same row with different superscripts are significantly different ($p \leq .05$).

^{x,y}Means listed in the same row with different superscripts are tended to be different ($p \leq .10$).

^c $n = 6$ replicates/treatment.

Table 5. Effects of dietary oregano powder supplementation on carcass yield and meat quality of broiler chicks^a.

Items	CTR	ATB	OP	SEM	p-value
Carcass yield					
R_{bm} , %	15.26	14.35	15.40	0.52	.37
R_{tm} , %	10.86	10.98	11.42	0.22	.24
Meat quality					
Cooking loss, %	4.86	6.62	5.72	1.12	.56
Dripping loss, %	20.04	18.18	18.97	1.25	.62
Shear force, N	14.23	14.53	13.47	1.39	.87
pH _i	6.34	6.43	6.40	0.06	.61
pH _u	5.77	5.77	5.73	0.04	.77
a^*_i	3.91	3.18	3.99	0.67	.66
a^*_u	5.29	4.85	5.13	0.62	.88
b^*_i	9.55	10.35	10.81	0.50	.23
b^*_u	11.36	12.98	12.57	0.71	.29
L^*_i	45.53	44.84	45.39	1.09	.90
L^*_u	49.77	51.79	51.41	0.99	.34

ATB: antibiotic; CTR: basal diet without additive; OP: oregano powder; a^* : redness; b^* : yellowness; L^* : lightness; R_{bm} : weight of breast muscle (g)/weight of eviscerated carcass (g); R_{tm} : weight of thigh muscle (g)/weight of eviscerated carcass (g).

pH_i, a^*_i , b^*_i and L^*_i were measured just after slaughtering (initial) and pH_u, a^*_u , b^*_u and L^*_u were measured 24 h later (ultimate).

^a $n = 6$ replicates/treatment.

activity was not affected by the dietary supplementation ($p > .05$). In addition, as shown in Table 5, the oregano powder supplementation did not significantly affect the carcass yield and meat quality of broiler chicks at 42 days of age ($p > .05$).

Discussion

The objective of our study was to determine whether OP added to the diets of broiler chicks would improve growth performance, immune and antioxidant status and meat quality. Before discussing the growth performance results of this paper further, it would be helpful to consider how phytochemical sources, like oregano, function as a growth promoter in comparison with antibiotics. In terms of mechanism it seems that the explanation of how antibiotics enhance or mediate animal growth performance is mainly based on a

hypothesis from the results of germ-free animal experiments that antibiotics affect the microbiota in animal intestine (Dibner & Richards 2005; Niewold 2007). However, this kind of explanation does not seem valid because antibiotics are used in a low and sub-therapeutic dosage in poultry and livestock, which considerably differs their intestine microbiota. Another potential explanation is that antibiotics normally accumulate in inflammatory cells, and most accumulated antibiotics enhance the intracellular killing of bacteria and attenuate the inflammatory response, thus influence animal production (Niewold 2007). Moreover, there is another hypothesis to explain the growth-promoting effect of antibiotics that the nutrient absorption is improved as the intestinal wall of treated animal is thinner in intestinal villi and total gut wall (Dibner & Richards 2005). In terms of phytogenic sources, the mechanism for functioning as a growth promoter has not yet been thoroughly investigated, but from previous research (Windisch et al. 2008; Hashemi & Davoodi 2010) the growth promoting effect of phytogenic sources is probably due to its antimicrobial activity. The mechanism by which oregano extract has antimicrobial effects *in vitro* was already investigated and reported (Krishan & Narang 2014). However, many previous studies indicated that oregano extract can act as a natural antioxidant, thus it would be also important and interesting to investigate the interaction between the growth and systemic antioxidative defence property by the OP supplementation.

In this study, OP showed marked growth performance advance in the grower period and the whole period of the trial, and interestingly, there was no difference relating to the growth between OP chicks and the broilers fed antibiotic. Many previous studies on chicken reported that oregano supplementation had the growth promoting effects (Chen et al. 2007; Malayoğlu et al. 2010; Roofchae et al. 2011). In contrast, others research papers indicated that there was no effect of oregano or its ingredients on broiler growth (Barreto et al. 2008; Avila-Ramos et al. 2012; Kirkpınar et al. 2014). In this study, the concentration of oregano was 150 mg/kg. Chen et al. (2007) also reported the growth promoting effects of oregano at this concentration but their paper did not show whether growth promoter function is affected by an increase in concentration. Roofchae et al. (2011) investigated the effect of oregano on broiler's growth performance at different concentrations from 300 mg/kg to 1200 mg/kg but it revealed the effect was higher at 600 mg/kg rather than 1200 mg/kg. In addition, some previous research indicated that there was no positive effect on growth performance at any

concentrations of oregano (Karimi et al. 2010). In the present study, the potential improvement in growth performance of the OP chicks may be due to a modulated health status, as evidenced by the improved systemic antioxidative capacity, although all the birds were fed under a standard condition. In our study, the lack of effect on immune status might be attributed to the environment in which the broilers were housed may not have required the broilers' immune system to develop sufficiently because of the clean environment (Florou-Paneri et al. 2006). The result of the lymphoid organs in this trial is in agreement with the observations by others authors that there was no significant effect of oregano on immunity status (Alp et al. 2012; Hashemipour et al. 2013). Moreover, no dietary effect on immune organs was observed in the present study, likely because those organs are less sensitive than others parameter such as plasma lysozyme for healthy chicks (Samuel et al. 2015).

Antioxidants in blood, cells and tissue fluids play an important role in neutralising the normal level of oxidative damage caused by the accumulated ROS (Saleh et al. 2010). The malondialdehyde (MDA) content reflects the antioxidant and lipid peroxidation status of cultured cells and animal tissues (Efe et al. 1999). Serum T-AOC is mainly regarded as the representation of the *in vivo* balance between oxidising species and antioxidant compounds and may give more biologically relevant information than that obtained from measuring concentrations of individual antioxidants (Ghiselli et al. 2000). In this study, we found that OP supplementation improved antioxidant status mainly through reducing MDA content and increasing the T-AOC in the serum of broiler chicks. From the antioxidant activity analysis of serum, it is likely that oregano supplement has some antioxidant activities, which may be triggered by some chemical compounds contained in oregano. This is seemingly caused as the numerous polyphenols in oregano scavenge radicals in blood. Marcinčák et al. (2008) and Hashemipour et al. (2013) indicated a positive effect of oregano on antioxidant activity of broiler chicks. However, Young et al. (2003) and Roofchae et al. (2011) reported no effect of oregano on antioxidant activity. Some antioxidants directly scavenge radicals but may not function as electron donors for superoxide dismutase. In this study, we did not observe a significant effect of OP on SOD activity, which may be due to the concentration the chemical compounds in OP. Previous research (Hashemipour et al. 2013) using carvacrol and thymol, the main monoterpenoids bioactive compounds of oregano essential oil, at higher concentration between 60 mg/kg and 200 mg/kg, carvacrol and thymol supplementation improved SOD

activity in serum depending on concentration. The concentration of carvacrol and thymol in the oregano powder used in this trial was 8.06% and 1.50%, respectively, as provided by the supplier.

In the present study, neither ATB nor OP had positive effect on carcase yield and meat quality of broiler chicks. Although many studies have been conducted with oregano in poultry diets, the results obtained from these studies were not consistent. Kirkpınar et al. (2014) reported that dietary oregano supplementation did not affect pH, L^* and b^* value of breast muscle at 300 mg/kg but it lowered a^* value compared with the control group. Hone et al. (2012) found no differences in breast or thigh muscle L^* , a^* or b^* values with supplemented oregano and anise essential oils and citrus peel powder. However, Park et al. (2015) observed that dried oregano powder reduced the cooking loss in the breast muscle of ducks. The lack effect of OP on meat quality in this study may be due to herb selected, basal diet, dose administered, environment conditions and the possible interactions with rearing conditions and/or diet ingredients.

Conclusions

The results of this paper, in some respects, still appear inconsistent with the previous research. However, the impact of oregano has not yet been fully understood since it is a mixture of an immense number of chemical compounds rather than a single chemical substance. The complexity of oregano's make-up may account for the ongoing uncertainties about various aspects of the effect of oregano, such as how oregano promotes animal growth and if it acts as an antibiotic agent *in vivo*. Further research will probably have to focus either on the mechanism by which oregano affects animal nutrition as a mixture of chemical compounds or on the side effect of oregano extracts rather than considering how it directly affects antioxidant status and improves animal growth.

In conclusion, our observations suggest that the oregano powder supplementation could improve the growth performance and antioxidant status of broiler chicks, and the favourable effects mainly occurred in grower phase in the broilers. The broiler chicks offer a useful animal model to study the growth promoting and antioxidant effects of oregano powder. More work on oregano powder application may benefit the broiler industry.

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